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U.S. PATENT APPLICATION

for

SPECIMEN HOLDER FOR A HIGH-PRESSURE FREEZING DEVICE

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SPECIMEN HOLDER FOR A HIGH-PRESSURE FREEZING DEVICE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This invention claims priority of the German patent application 100 65 143.7 which is incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The invention concerns a specimen holder for a high-pressure freezing device.

BACKGROUND OF THE INVENTION

[0003] For purposes of the invention, a "high-pressure freezing device" is understood to be a freezing system for rapid freezing (vitrification) of water-containing specimens under high pressure. Devices of this kind are described in DE Patent 18 06 741, EP 0 853 238 A1, and EP 0 637 741 A1.

[0004] A high-pressure freezing device of this kind is being successfully marketed by the Applicant under the name "Leica EM HPF," and is depicted in the document "LEICA EM HPF, High Pressure Freezer, 1.K.-LEICA EM HPF-E-6/94, Juni 1994."

[0005] The "Leica EM HPF" high-pressure freezing device makes it possible to vitrify conventional specimens under a pressure of approximately 2000 bar at a cooling rate of 10^3–10^5 K/s. With this known unit, the critical cooling phase from room temperature to -100°C lasts approximately 10 ms at the surface of the specimen (cooling rate of 10^4 K/s).

[0006] The cooling rate in the interior of the specimen depends exclusively on the physical properties of the specimen. In order to

vitrify thicker biological or water-containing specimens, the specimen is exposed to high pressure.

[0007] Because of the elevation in pressure, a slower cooling rate is sufficient for vitrification. The cooling rate for vitrification of biological specimens is approximate 10^5–10^6 K/s at standard pressure, but at 2000 bar the cooling rate is only 10^3–10^4 K/s; in other words, under high pressure biological specimens can still be vitrified at cooling rates that are about a hundred times lower.

[0008] Metal holders that comprise at least two shaped parts detachably joinable to one another are used for freezing the specimens, the shaped parts joined to one another forming a chamber for reception of the specimens. Specimen holders of this kind are depicted in the aforementioned document "LEICA EM HPF, High Pressure Freezer, 1.K.-LEICA EM HPF-E-6/94, Juni 1994" and in the document "Balzers, Hochdruck-Gefriermaschine [High-pressure freezing machine] HPM 010, Balzers Union Aktiengesellschaft, no publication date."

[0009] In order to freeze the specimen, the specimen holder is clamped in the high-pressure freezing device in such a way that on the one hand specimen loss is prevented, and on the other hand a sufficient portion of the surface remains freely accessible for a cryogen, e.g., liquid nitrogen, to be sprayed onto it. An arrangement of this kind is depicted and described in unpublished DE 100 15 773.

[0010] From the known art of refrigeration and from the physical processes, it is also known that the cooling rate at the center of a biological specimen is determined only to a limited extent by the cooling rate at the surface. The essential feature in this context is that with rapid cooling rates at the surface, a kind of "saturation effect" occurs, whereas lower cooling rates at the surface inevitably lead to poor freezing results. It is therefore essential that the cooling rate

achievable by a unit be transferred in as undiminished a fashion as possible by the specimen holding system onto the surface of the biological specimen.

SUMMARY OF THE INVENTION

[0011] It is therefore an object of the present invention to create a specimen holder for a high-pressure freezing device that transfers the achievable cooling rate in as undiminished a fashion as possible onto the surface of the specimen.

[0012] The present invention provides for a specimen holder for water-containing specimens for high-pressure freezing, the specimen holder comprising at least two shaped parts detachably joinable to one another, wherein the joined shaped parts form a receptacle for holding a specimen, wherein at least one of the shaped parts comprises a diamond. Diamond possesses not only extreme hardness but also the advantage of low specific heat and very good thermal conductivity.

[0013] In a further embodiment of the invention, at least one of the shaped parts, preferably the shaped part fabricated of diamond, is of disk-shaped configuration. A spacer ring fabricated from metal, which determines the depression necessary for specimen reception, is provided between the two shaped parts. The spacer ring can also assume the sealing function in this context.

[0014] Provision can, of course, also be made for only one of the shaped parts to be fabricated of diamond or for the shaped part to comprise a mounted diamond. In this case the depression for specimen reception can be accomplished by the mount of the diamond or by a corresponding configuration of the other shaped part. A preferred exemplary embodiment is a specimen holder having one disk-shaped shaped part made of diamond, and one disk-shaped one made of

metal. The metal shaped part has a circumferential shaped-on bead at the rim.

[0015] In a further embodiment of the specimen holder, the diamond or one of the shaped parts can be equipped with an orifice for the delivery of high pressure.

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[0016] It has proven to be advantageous in the context of such specimen holders to use gold or aluminum or copper as the metal, and to utilize a commercially available polycrystalline CVD industrial diamond as the diamond.

[0017] In a further embodiment of the invention, both shaped parts are of planar configuration on their inwardly directed surfaces. A spacer running around the surfaces is arranged between those surfaces as a seal and in order to constitute the specimen receptacle. Differently dimensioned receptacles for the specimens can easily be produced by way of differently dimensioned spacers.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The invention is depicted and explained in more detail, in several exemplary embodiments, on the basis of the schematic drawings in which:

[0019] Fig. 1 shows a sectioned depiction of the specimen holder having two shaped parts manufactured from diamond and a circumferential metal ring;

[0020] Fig. 2 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and one shaped part made of metal;

[0021] Fig. 3 shows a sectioned depiction of the specimen holder having two shaped parts made of diamond and an additional shaped part with a high pressure delivery line;

[0022] Fig. 4 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and another shaped part with a high pressure delivery line; and

[0023] Fig. 5 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and a shaped part forming a sample receptacle and a seal.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Fig. 1 shows a specimen holder 1 having a shaped part 2 and a shaped part 3 arranged opposite each other. Both shaped parts 2 and 3 are produced from diamond and are configured as disk-shaped plates. A spacer ring 9, which preferably is made of metal, is provided between the two shaped parts 2 and 3. The metal used here is preferably gold, copper, or aluminum. The size of spacer ring 9 determines the size of sample receptacle 6. It is thus easy to achieve sample receptacles 6 of different sizes by way of differently dimensioned spacer rings 9.

[0025] Specimen receptacle 6 is isolated via spacer ring 9 when clamped into a clamping device (not depicted) of a high-pressure freezing device.

[0026] Fig. 2 shows a further exemplary embodiment of specimen holder 1 having one shaped part 2 fabricated of diamond and an oppositely arranged shaped part 3 produced from metal. Shaped part 3 has, at its rim, shaped-on beads 8 extending around the rim that assume the function of spacer ring 9 of Fig. 1. Specimen receptacles 6 of different sizes can be implemented by different dimensioning of beads 8. A fixed specimen receptacle 6 is constituted by shaped-on beads 8, thereby facilitating insertion and removal of the specimen.

[0027] Fig. 3 shows a specimen holder 1 corresponding to the embodiment of Fig. 1, where an orifice 10 for a downstream high-

pressure device (not depicted here) is provided in shaped part 3. A further shaped part 4 having a high-pressure conduit 5 is associated with shaped part 3. High-pressure conduit 5 corresponds to orifice 10, which terminates in specimen receptacle 6. The necessary pressure can thereby be generated directly in specimen receptacle 6.

[0028] Fig. 4 shows an exemplary embodiment of specimen holder 1 corresponding to Fig. 3, where shaped part 4 is directly joined to shaped part 2 via seal 9. In this exemplary embodiment, shaped part 3 has been completely removed.

[0029] Fig. 5 shows an exemplary embodiment of specimen holder 1 according to Fig. 4, where the encircling spacer ring 9 for sealing has been replaced by metal shaped part 3 having a bottom 7 and circumferentially shaped-on beads 8. This shaped part 3 corresponds to shaped part 3 of Fig. 2, and bottom 7 is very thin. Since shaped part 3 is arranged removably in specimen holder 1, shaped part 3 can be removed separately. It is thus easier for the specimens to be introduced into and removed from specimen holder 1 or specimen receptacle 6.

[0030] The shaped parts described above and fabricated from diamond, preferably from a polycrystalline CVD diamond, may comprise one or more mounted diamonds.

[0031] The external surfaces of the shaped elements may be irregularly shaped, thereby constituting a greater surface area for spraying on liquid nitrogen.

PARTS LIST

1 Specimen holder
2 Shaped part
3 Shaped part
4 Shaped part
5 High-pressure conduit
6 Specimen receptacle
7 Bottom
8 Bead
9 Spacer ring
10 Orifice